CHROMOSOMAL HOMOLOGIES BETWEEN *ANOPHELES NOROESTENSIS* AND CERTAIN OTHER ANOPHELINES[1]

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ABSTRACT. The salivary chromosome map of *Anopheles noroestensis* is presented and proposed as the standard for this species. The complement consists of 5 paired elements: a telocentric X-chromosome, right and left arms of submetacentric chromosome 2 and right and left arm of metacentric chromosome 3. The banding pattern is compared with several other species of the subgenus *Nyssorhynchus*. The highest level of homology is with members of the *argyritarsis* series, a slightly lower level with *An. aquasalis* and *An. nuneztovari*, and the least homology with *An. albimanus*.

INTRODUCTION

Homologies in the banding patterns in the salivary gland chromosomes of a number of anophelines of the subgenus *Nyssorhynchus* Blanchard have been reported. All studies indicate similarities of the free and centromere ends of the autosomes (Kitzmiller and Chow 1971, Keppler et al. 1973, Kitzmiller et al. 1973), and some studies report more extensive homologies (Kreutzer et al. 1972, 1975). The cytogenetic data show that morphological similarity correlates with chromosomal banding homology. These and other studies (Kitzmiller 1976) have reported that with few exceptions each species can be identified by the distinct pattern in the X-chromosome. The chromosomes of *Anopheles nuneztovari* Gabaldon, which is morphologically similar to *An. noroestensis* Galvao and Lane (Gorham et al. 1967), have been described (Kitzmiller et al. 1973). This study assesses the level of chromosomal banding relationship between *An. noroestensis* and *An. nuneztovari* and certain other species in the subgenus *Nyssorhynchus*.

MATERIALS AND METHODS

The larvae used to prepare the chromosome map of *An. noroestensis* were collected in the states of Guanabara and Rio de Janeiro, Brazil. Slides were made following the method described by French et al. (1962). The "dry-ice" method was used to make the slides permanent. Detailed observations of the banding pattern were made at 1000 X using a Zeiss phase contrast system. The chromosome complement is shown in figure 1, and the proposed salivary gland chromosome map is shown in figure 2. Figure 3 is a photographic map of the paired salivary gland chromosome arms.

DESCRIPTION OF THE CHROMOSOMES. As in other mosquitoes the diploid chromosome number is 6, 2 pairs of autosomes and 1 pair of sex chromosomes (Guedes et al. 1957). The males are heterogametic. The telocentric X chromosome averages 70 micra in length, the right arm of the submetacentric chromosome (2R) averages 200 micra, the left arm (2L), 141 micra, and the right (3R) and left (3L) arms of metacentric chromosome three each average 139 micra. These chromosome lengths are similar to those of several other *Nyssorhynchus* species. The numbering system for the arms is the same as in the *An. darlingi* Root (Kreutzer et al. 1972) system: X-chromosome, zones 1 through 5; 2R, zones 6 through 15; 2L, zones 16 through 25; 3R, zones 26 through 35; 3L, zones 36 through 45.

[1] This paper is dedicated to our colleague and friend, Dr. M. G. Rabbani who shared in the field work reported in this paper. Dr. Rabbani died of drug resistant *falciparum* malaria in Manaus, Brazil in January 1977 while studying malaria transmission by *Anopheles darlingi*.

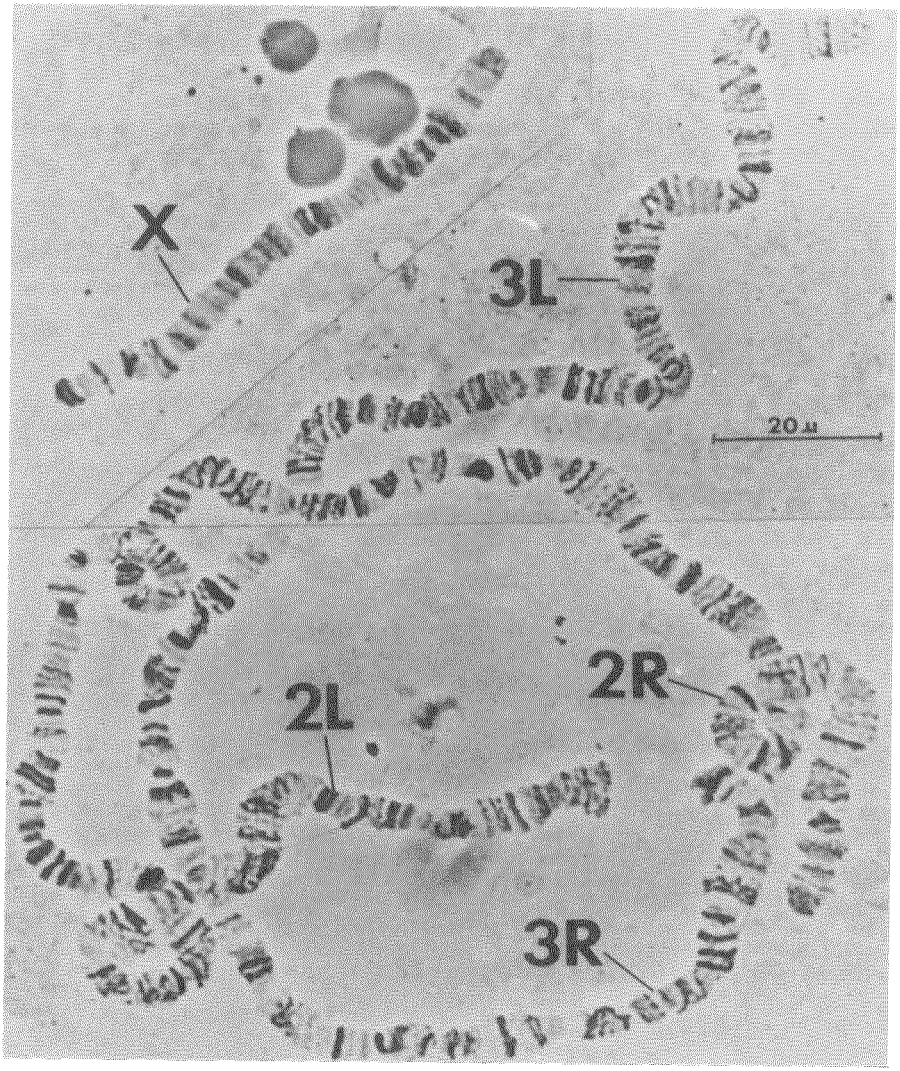
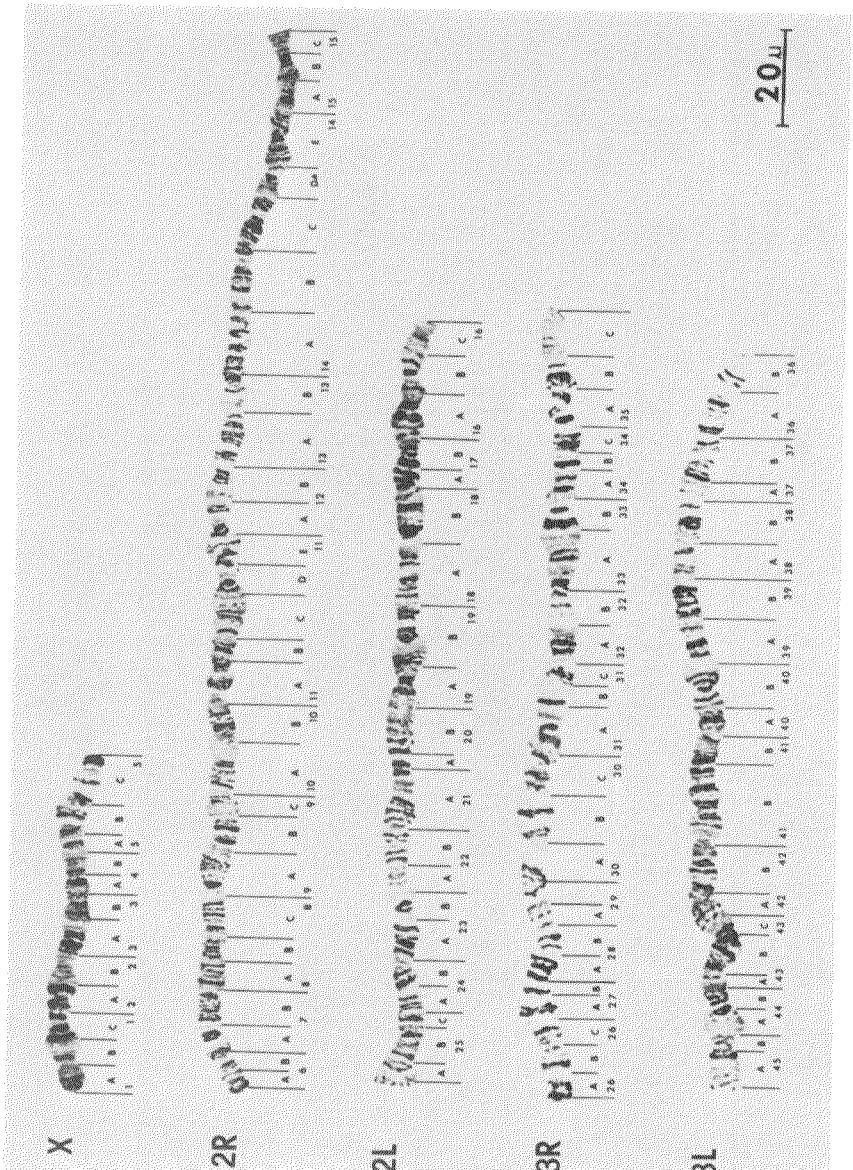


Fig. 1. *Anopheles noroestensis*, salivary gland chromosomes.


X
2R
2L
3R
3L
10μ
1
5
6
26
15
35
16
36
25
45
ANOPHELES
NOROESTENSIS
SALIVARY
CHROMOSOME
MAP


X
2R
2L
3R
3L
20 µ

PRINCIPAL RECOGNITION AREAS. X Chromosome. The telocentric X chromosome is the shortest element of the complement, and has no apparent banding similarity with any other anopheline. A series of what appear to be 5 widely spaced dark bands in 1C and 2A is characteristic of the free end of the chromosome. These bands are followed in 3A by 4 more closely spaced dark bands, the second of which is thickest. In 5B and 5C there is a series of widely spaced dark bands which end at the centromere with a pair of close dark bands.

Chromosome 2, right arm. The free ends and centromere regions of this and other autosomal arms have sections which are homosequential with other species in the subgenus. The puff in 7A which ends with 2 thick dark bands is characteristic of the free end of 2R. The center of the arm, 11E-12B, contains a series of bands which begins in 11E with a pair and then a single dark band, followed in 12A by 2 closely spaced dark bands, the second in a constriction; then 2 widely spaced thick dark bands at the end of 12A and beginning of 12B, and ends with 4 closely spaced dark bands at the end of 12B. The thick dark bands flanked by lighter bands in 15A and 15B are characteristic of the centromere region of the arm.

Chromosome 2, left arm. The five widely spaced dark bands in 25A-25C are characteristic of the free end of 2L. The series of dark bands in 21A-20B flanked by areas which contain lightly staining bands is diagnostic of the center of this arm. Near the centromere in region 16B there are 3 widely spaced dark bands, the center one thicker.

Chromosome 3, right arm. The puff in 27A which contains a wide, broken, dark staining band followed by a thin dark band is characteristic of the free end of the arm. The thick dark bands in 30C and 31A are landmarks in the center of the arm. In 35B the series of 3 dark bands flanked by areas which contain lightly stained bands is indicative of the centromere region of the arm.

Chromosome 3, left arm. The free end of 3L, 45A-44A, is characterized by the dark band at the end of 45A which is flanked by areas with lightly staining bands. The series of bands in 40B, the center 3 dark and the outer ones light, marks the center of the arm. The 2 widely spaced dark bands in 36B flanked by lightly staining areas indicate the centromere region of 3L.

Inversions. Four naturally occurring inversions, 2 in 2R and 1 each in 3R and 3L have been recovered from these samples of *An. noroestensis*. A short inversion at the free end of 2R, 2R (a/+) of region mid 6A-mid 7B, has been recovered only once as a heterozygote, and the second 2R inversion, 2R (b/+) in regions 13A-14D, has a frequency of 7%. The inverted 3R sequence, 3R (a/+) in regions in 27B-32B, has been recovered at a frequency of 24% and the inverted 3L sequence, 3L (a/+) in regions 39A-38B, has a frequency of 3%. As has been reported in previous studies the breakpoints of the inversions are located in regions where the bands are light and broken. The notation (a/+) indicates an inversion which may exist as a heterozygote or as either homozygote.

DISCUSSION

An. noroestensis is morphologically more similar to *An. nuneztovari* than to *An. albitarsis* but the salivary gland chromosome banding patterns indicate at least an equal if not a slightly closer homology with *An. albitarsis* (Kreutzer et al. 1976). For this reason basic banding comparisons are made between *An. noroestensis* and *An. albitarsis*. As in other studies of anopheline chromosomes the banding pattern in the X chromosome is distinctive (Kitzmiller et al. 1967); therefore, this element of the complement can be used for species identification. In 2R regions 6A-7B at the free end and 15A-15C at the centromere end of the arm are similarly banded in *An. noroestensis* and in *An. albitarsis*. The banding pattern in the center of this arm in *An. noroestensis* as well as the center sections of the other autosomal arms are distinctive and show little similarity with the pattern

found in *An. albitarsis*. If region 25C-23A of *An. noroestensis* is inverted a long section at the free end of 2L is homosequential with *An. albitarsis*. At the centromere end of 2L, 16 A-16C is identical in both species. The free end of 3R in *An. noroestensis*, 26A-27A, is somewhat similar to *An. albitarsis*, but is identical with the same region in *An. nuneztovari*. Regions 35B-35C at the centromere are identical in both *An. noroestensis* and *An. albitarsis*. In 3L regions 45A-44A at the free end and 36A-36B at the centromere end are identical in both species. These identically banded regions in *An. noroestensis* and *An. albitarsis* have been drawn and numbered in the *An. noroestensis* map as they are shown in the *An. albitarsis* map.

In addition to the banding similarities to *An. albitarsis* the *An. noroestensis* chromosomes have homologies at the free and centromere ends of the autosomes with *An. nuneztovari*, *An. aquasalis* Curry (Kitzmiller and Chow 1971), *An. albimanus* Wiedemann (Keppler et al. 1973), *An. darlingi*, and *An. argyritarsis* Robineau-Desvoidy (Kreutzer et al. 1975). These relationships are summarized in Table 1 which estimates the amount of similarity between given regions in *An. noroestensis* and several closely related species.

Four inversions have been recovered from these samples of *An. noroestensis*. Two have breakpoints which indicate relationships with other species. One of the inversions, 2R (b/+), of *An. noroestensis* and one in *An. albitarsis* have common breakpoints at 14E near the centromere end of the arm. The other, in 3R at 35A, is also one breakpoint for naturally occurring inversions recovered from populations of both *An. albitarsis* and *An. darlingi*. It also marks the start of the region near the centromere that is homosequential in all of the species of *Nyssorhynchus* thus far studied.

The amount of chromosomal relationship between *An. noroestensis* and the other species discussed here is shown in Table 2.

Table 1. Similarities between *An. noroestensis* and several other species in the banding patterns of regions indicated. H = homosequential; ++ = considerable similarity; + = some similarity.

	darlingi	*nuneztovari*	*aquasalis*	*albitarsis*	*albimanus*	*argyritarsis*
6A-7B	H	+	++	H	+	H
15A-15C	H	H	H	H	H	H
16A-16C	H	H	H	H	H	H
25A-23A	H	+	++	H	+	H
26A-27A	+	H	+	+	+	+
35B-35C	H	H	H	H	H	H
36A-36B	H	H	H	H	H	H
45A-44A	H	+	H	H	H	H

Table 2. Percent of each arm, per species, homosequential with *An. noroestensis*.

	albitarsis darlingi argyritarsis	*aquasalis*	*nuneztovari*	*albimanus*
X	0	0	0	0
2R	.24	.20	.10	.10
2L	.43	.40	.20	.20
3R	.10	.10	.38	.10
3L	.20	.20	.15	.20

Figures refer to the percentage of the total length of each arm, per species, which is homosequential with *An. noroestensis.*

The greatest level of similarity is among the 3 species in the *An. argyritarsis* series, slightly less with both *An. aquasalis* and *An. nuneztovari.* The level of banding homology with *An. albimanus* indicates a more distant cytogenetic relationship.

Literature Cited

French, W. L., R. H. Baker and J. B. Kitzmiller. 1962. Preparation of mosquito chromosomes. Mosquito News 22:377–383.

Gorham, J. R., C. J. Stojanovich and H. G. Scott. 1967. Clave ilustrada para los mosquitos Anofelinos de Sudamerica Oriental. U.S.P.H.S., C.D.C., Atlanta, Georgia, pp. 1–64.

Guedes, A. E., E. M. Amorim and G. Schreiber. 1957. Analise Dos Chromosomos Salivares Em Anofelinos Brasileiros. Rev. Bras. de Malar. e Doen. Trop. 9:247–250.

Keppler, W. J., Jr., J. B. Kitzmiller and M. G. Rabbani. 1973. The salivary gland chromosomes of *Anopheles albimanus.* Mosquito News 33:42–49.

Kitzmiller, J. B. 1976. Genetics, Cytogenetics and Evolution of Mosquitoes. Adv. in Genetics 18:315–433.

Kitzmiller, J. B., G. Frizzi, and R. H. Baker. 1967. Evolution and speciation within the *maculipennis* complex of the genus *Anopheles. In:* Genetics of Insect Vectors of Disease, Wright and Pal (Eds.) Elsevier Publishing Co., Amsterdam. Chapter 5:151–210.

Kitzmiller, J. B. and G. Y. Chow. 1971. The salivary gland chromosomes of *Anopheles aquasalis.* Rev. Brasil. de Malar. e Doen. Trop. 23:65–85.

Kitzmiller, J. B., R. D. Kreutzer and E. Tallaferro. 1973. Chromosomal differences in populations of *Anopheles nuneztovari.* Bull. Wld Hlth Org. 48:435–445.

Kreutzer, R. D., J. B. Kitzmiller and E. Ferreira. 1972. Inversion polymorphism in the salivary gland chromosomes of *Anopheles darlingi* Root. Mosquito News 32:555–565.

Kreutzer, R. D., J. B. Kitzmiller and M. G. Rabbani. 1975. The salivary gland chromosomes of *Anopheles argyritarsis* compared with those of certain other species in the subgenus *Nyssorhynchus.* Mosquito News 35:354–365.

Kreutzer, R. D., J. B. Kitzmiller and M. G. Rabbani. 1976. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis.* Acta Amazonica 6:473–481.